

A Research Note

EFFECT OF INCUBATION TEMPERATURE ON ACID AND FLAVOR PRODUCTION IN MILK BY LACTIC ACID BACTERIA¹

S. M. DUTTA, R. K. KUILA, B. C. ARORA AND
B. RANGANATHAN

ABSTRACT

The effect of incubation temperature on acid and flavor production by 8 lactic starter cultures was studied. Higher titratable acidity was produced at 22 than at 30 C by *Streptococcus cremoris*, while a reverse trend was observed with *Streptococcus diacetilactis*. With *Streptococcus thermophilus*, *Lactobacillus casei*, and *Lactobacillus helveticus*, increasing the incubation temperature from 37 to 42 C retarded acid production by 8.54, 31.8, and 37.4%, respectively. Volatile acidity increased at higher incubation temperatures in some cultures, while in others, a significant decrease was observed. Higher diacetyl production was noted when *S. diacetilactis* was incubated at 22 C, as compared to 30 C, while the reverse occurred with *S. thermophilus*. Acetoin production by *S. diacetilactis* was enhanced 7-fold by increasing the incubation temperature from 22 to 30 C. No significant difference was noted in proteolytic activity of all cultures, regardless of incubation temperature.

Among the factors that affect growth and activity of starter cultures, incubation temperature is known to play an important role (9). Hammer and Babel (8) stressed the need of an optimum incubation temperature for maintaining a proper balance between acid producing and aroma bacteria. The present communication reports the effect of incubation temperature on biochemical changes produced in milk by selected lactic cultures.

EXPERIMENTAL

Eight cultures including *Streptococcus lactis* (C-10), *Streptococcus cremoris* (C-1), *Streptococcus diacetilactis* (DRC-1), *Streptococcus thermophilus* (HST), *Lactobacillus bulgaricus* (LBW), *Lactobacillus casei* (L-8), *Lactobacillus helveticus* (L-23), and *Lactobacillus plantarum* (L-24) were studied.

Reconstituted nonfat milk steamed for 30 min was used. Streptococci were incubated at 22 and 30 C, while lactobacilli were incubated at 37 and 42 C. The incubated milk samples were analysed after 24 and 48 hr. Total acidity was determined by titrating 10 g of sample with 0.1 N NaOH. Volatile acidity was determined by the method of Hempenius and Liska (10) by titrating 100 ml of distillate from 50 g of the sample with 0.01 N NaOH. Diacetyl was determined by Owades and Jacovac's method as modified by Pack et al. (13), while acetoin was measured by the method of Anderson and Leesment (1). Proteolytic activity was de-

termined by Hull's method (11) and expressed as milligrams of tyrosine liberated per gram of the sample.

RESULTS AND DISCUSSION

Although the role of incubation temperature on acid and flavor production by starter cultures has been studied by several workers (2, 14, 15) a wide range of incubation temperatures, viz. 22, 30, or 37 C, have been reported to be optimum for acid production (2, 3, 6, 9). Golding et al. (7) reported that propagation at 21-22 C gave an active starter, though the optimum temperature for acid development by starters was 30 C. Variation in acid production by starter cultures in response to incubation temperature is evident from the data presented in Tables 1 and 2. Whereas *S. cremoris* produced higher titratable acidity at 22 than at 30 C, the reverse occurred with *S. diacetilactis*. The higher incubation temperature of 42 C retarded acid production by *S. thermophilus*, *L. casei*, and *L. helveticus*, while with *L. bulgaricus*, increased titratable acidity was noted at that temperature. More volatile acids were produced at higher incubation temperatures by *S. lactis*, *S. cremoris*, and *S. thermophilus*. Folda (5) reported similar observations with single and mixed strain starters. In normal commercial practice in India and elsewhere, the cooking temperature during cheese manufacture is about 40 C which is appreciably higher than the optimum incubation temperature for growth and activity of starter cultures. This results in a slow rate of acid production during cooking of the cheese curd. Further, increased amounts of volatile acids are produced at higher incubation temperatures. These observations suggest the need for selecting cheese starters with high temperature optima for uniform acid development and improved flavor production during cheese ripening. In the yogurt starters, namely *L. bulgaricus* and *S. thermophilus*, the higher titratable acidity by the former and increased total and volatile acid production by the latter at 42 C may be viewed with interest, since the above incubation temperature is normally used during the manufacture of yogurt.

TABLE 1. EFFECT OF DIFFERENT INCUBATION TEMPERATURES ON ACID AND FLAVOR PRODUCTION IN MILK BY SELECTED STREPTOCOCCI¹

	<i>Streptococcus lactis</i>		<i>Streptococcus cremoris</i>		<i>Streptococcus diacetylactis</i>		<i>Streptococcus thermophilus</i>	
	22 C	30 C	22 C	30 C	22 C	30 C	37 C	42 C
Total acidity (per cent lactic)	0.85	0.86	1.00	0.92	0.80	0.90	0.82	0.75
Volatile acidity (ml of 0.01 N NaOH/50 g of curd)	6.4	8.2	6.0	8.4	22.5	22.7	5.0	7.3
Diacetyl (ppm)	0.00	0.00	0.00	0.00	25.0	14.0	6.0	12.0
Acetyl methyl carbinol (ppm)	0.00	0.00	0.00	0.00	13	87	0.00	0.00
Proteolytic activity (mg of tyrosine liberated/g of curd)	0.25	0.27	0.40	0.41	0.37	0.42	0.16	0.17

¹Cultures were grown in reconstituted nonfat milk and examined after 18 hr. Results represent an average of three trials.

TABLE 2. EFFECT OF DIFFERENT INCUBATION TEMPERATURES ON ACID AND FLAVOR PRODUCTION IN MILK BY SELECTED LACTOBACILLI¹

	<i>Lactobacillus bulgaricus</i>		<i>Lactobacillus casei</i>		<i>Lactobacillus plantarum</i>		<i>Lactobacillus helveticus</i>	
	37 C	42 C	37 C	42 C	37 C	42 C	37 C	42 C
Total acidity (per cent lactic)	1.40	1.85	0.22	0.15	0.25	0.23	0.24	0.15
Volatile acidity (ml of 0.01 N NaOH/50 g of curd)	23.0	26.0	6.5	4.5	6.8	7.4	4.8	2.5
Proteolytic activity (mg of tyrosine liberated/g of curd)	0.38	0.42	0.15	0.18	0.19	0.20	0.17	0.18

¹Cultures were grown in reconstituted nonfat milk and examined after 18 hr. Results represent an average of three trials. These cultures did not produce diacetyl or acetoin in milk.

As regards incubation temperature and flavor production by starter cultures, reducing it from 30 to 22 C enhanced diacetyl production by *S. diacetylactis*, while the reverse was true of *S. thermophilus*. In the former organism, a 7-fold increase in acetoin production was observed by incubating at 30 rather than at 22 C. Pack et al. (14) observed earlier initiation of diacetyl synthesis as well as better growth at 30 C by a strain of *S. diacetylactis*. Increased accumulation of diacetyl by *S. thermophilus* at 42 C noted in these results may be considered significant since this culture has been reported as one of the predominant streptococci occurring in dahi samples in almost all parts of India (12). No significant differences were noted in proteolytic activity of all cultures, regardless of incubation temperature.

ACKNOWLEDGEMENT

The authors express their thanks to the U.S. Department of Agriculture, Agricultural Research Service, Eastern Re-

gional Office, American Embassy, New Delhi for generous financial assistance from PL 480 funds.

REFERENCES

1. Anderson, I. and H. Leesment. 1962. Wachstumsverhältnisse von *Str. diacetylactis* in Mischkulturen XVI Int. Dairy Congr. III(2):217.
2. Babel, F. J. 1946. Factors influencing acid production by cheese cultures. I. Effect of cooking temperature on acid production in the manufacture of Cheddar cheese. J. Dairy Sci. 29:589-596.
3. Dahlberg, A. C., and F. Ferris. 1945. Influence of the frequency of transfer of lactic starters upon rate of acid development and quality of Cheddar cheese. J. Dairy Sci. 28:771-778.
4. Dolezalek, J. 1951. Factors affecting the diacetyl content of butter starter cultures. Sborn. Inst. Akad. zemed. 24:355-366.
5. Folda, W. 1952. Flavor development in starter. Milchwissenschaft 7:49-53.
6. Golding, N. S., H. Amundson, and R. O. Wagenaar. 1943. Factors affecting the development of acidity in pasteurized skim milk inoculated with commercial lactic starters. J. Dairy Sci. 26:909-919.
7. Golding, N. S., L. McCorkle, and F. Milich, 1951. Some

factors which may affect activity tests for lactic starters. Proc. W. Div. Amer. Dairy Sci. Ass. 36-46.

8. Hammer, B. W., and F. J. Babel. 1957. Dairy bacteriology. 4th ed. John Wiley & Sons Inc., New York.

9. Harrison, J., and D. V. Dearden. 1941. Slowness in cheese making. J. Dairy Res. 12:35-43.

10. Hempenius, W. L., and B. J. Liska. 1968. Method for determining volatile acids in cultured milk products. J. Dairy Sci. 51:221.

11. Hull, M. E. 1947. Studies on milk proteins. II. Colorimetric determination of the partial hydrolysis of the proteins in milk. J. Dairy Sci. 30:881.

12. Laxminarayana H., V. K. N. Nambudripad, V. Lakshminarasim, S. N. Anantharamiah, V. Sreenivasamurthy, and K. K. Iya. 1952. Studies on dahi. III. Taxonomy of lactic

acid bacteria of dahi. Ind. J. Vet. Sci. 22(I):27-49.

13. Pack, M. Y., W. E. Sandine, P. R. Elliker, E. A. Day, and R. C. Lindsay. 1964. Owades and Jacovac method for diacetyl determination in mixed strain starters. J. Dairy Sci. 47:981.

14. Pack, M. Y., E. R. Vedamuthu, W. E. Sandine, P. R. Elliker, and H. Leesment. 1968. Effect of temperature on growth and diacetyl production by aroma bacteria in single and mixed strain starter culture. J. Dairy Sci. 51:339-344.

15. Sasaki, R., T. Tsugo, T. Imamura, and M. Murai. 1953. Studies on the diacetyl production by lactic acid bacteria. Int. Dairy Congr. 4:611-613.

16. Sasaki, R., T. Tsugo, T. Imamura, and M. Murai. 1954. Studies on the diacetyl production by lactic acid bacteria. Jap. J. Zootech Sci. 24:176-179.